PATENT

**UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Bavykin, et al.

Title: COLUMN DEVICE FOR ISOLATION AND LABELING OF NUCLEIC ACIDS

Serial No.: 09/751,654

Filing Date: December 29, 2000

Examiner: Suryaprabha Chunduru

Art Unit: 1755

Attny Docket: 0003/00797



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## DECLARATION UNDER 37 C.F.R. 1.132

I, Sergei G. Bavykin, co-inventor of the invention claimed in United States Patent Application No.: 09/751,654, hereby declare that:

1. I am a Scientist at Argonne National Laboratory, Argonne, IL. I am currently a molecular biologist in the Biochip Technology Center at Argonne..

2. I received a PhD in Biology from Institute of Molecular Biology, Acad. Sci. of USSR, Moscow, USSR, in 1981. I received an M.D. in Medical Biophysics from Pirogov Moscow Medical Institute in 1975. I have been employed at Argonne since

1996. Prior to my employ at Argonne, I was a Visiting Scientist in the Molecular Biology Institute, University of California, Los Angeles from 1995 to 1996. From 1988 to 1995 I was a professor in the Engelhardt Institute of Molecular Biology, Acad. Sci. of Russia, Moscow. I was also a Visiting Scientist at University of California, Davis from 1992-93.

3. I am a co-inventor in at least three patent applications pending in the areas related to methods for labeling DNA and RNA, fluorescence microscopy, and microbiology.

4. I am an author or a co-author of at least 35 publications in the area of molecular biology, biochemistry, enzymology, and bioengineering. Exemplary publications include Applied Environmental Microbiology, Methods of Enzymology, Proceedings of National Academy of Sciences USA, Analytical Biochemistry, Journal of Biological Chemistry, Biochemistry, Journal of Molecular Biology, Nucleic Acids Research, and Proceedings Academy of Sciences USSR.

5. I am the author or co-author of the following publications:

*Radical generating coordination complexes as a tool for rapid and effective fluorescent labeling and fragmentation of DNA or RNA for microarray hybridization*, (submitted), 2002.

*Portable system for microbial sample preparation and oligonucleotide microarray analysis*, Appl. Eviron. Microbiol. 67, 922-928, 2001.

*Advances in DNA-protein crosslinking applications for chromatin studies in vitro and in vivo*, Methods Enzymol. 304, 516-533, 1999.

*Attachment to the nuclear matrix mediates specific alterations in chromatin structure*, Proc. Natl. Acad. Sci. USA 95, 14757-14762, 1998.

*Zero-length protein nucleic acid crosslinking by radical-generating coordination complexes as a probe for analysis of protein-DNA interactions in vitro and in vivo*, Anal. Biochem. 263, 26-30, 1998.

*Nucleosome structural transmission during chromatin unfolding is caused by conformational changes in nucleosomal DNA*,J. Biol. Chem. 273, 2429-2434, 1998.

*DNA-protein crosslinking studies: beyond the seemingly invariable nucleosome core structure*, Chemtracts Biochem. Mol. Biol. 10, 723-736, 1997.

*Chromatin studies by DNA-protein cross-linking*, Methods 12, 36-47, 1997.

*Alterations in nucleosome core structure in linker histone depleted chromatin*, J. Biol. Chem. 271, 3831-3836, 1996.

*Rearrangement of the histone H2A C-terminal domain in the nucleosome*, Proc. Natl. Acad. Sci. USA 91, 6845-6849, 1994.

*Mapping DNA-protein interactions by cross-linking*, Methods in Enzymology 170, 386-408, 1989.

*Primary organization of core particles reconstituted or isolated from chromatin*, Mol. Biiol. 22, 531-538, 1988.

*Primary organization of core particles is invariable in repressed and active nuclei from animal, plant and yeast cells*, Nucl. Acids Res. 13, 3439-3459, 1985.

*The method of crosslinking histones to DNA partly depurinated at neutral pH*, Anal. Biochem. 110, 93-101, 1981.

*Nucleosome primary organization: histone-DNA interactions*, Proc. Acad. Sci. USSR (Dokladi AN SSSR) 242, 715-718, 1978.

6. I am a coinventor in the above-identified patent application. I have studied the Proudnikov and Ekenberg prior art cited by the Examiner. Proudnikov and I were colleagues during his research at Argonne Laboratories. Generally, Proudnikov discloses a chemical method for fluorescent labeling of DNA and RNA, with only DNA manipulated in a column.

7. Proudnikov describes a method of labeling based on a depurination or an oxidation reaction. Proudnikov's method is not based on radical-mediated chemistry.

This is why his process takes 10 hours to complete. Proudnikov is based on the oxidation of furanose, after which a hydroxyl group is excised with $NaIO_4$ Proudnikov's reaction does not involve any radical generating groups whatsoever.

8. Proudnikov's process is aerobic. The inside of Proudnikov's column does not confer an anaerobic environ. In addition, it would make no sense for Proudnikov to establish anaerobic environs (and take the additional steps required) because its ionic chemistry works in the presence of oxygen. No special steps, such as profusing the solution or the reaction column with an inert gas, are taken in Proudnikov in order to purge oxygen from the reactants in Proudnikov.

9. Proudnikov's chemical reaction is much different than the chemical reaction used in my co-invented method of labeling DNA and RNA. Our method utilizes radical-based chemistry. Specifically, the nucleic acid is modified with radicals produced via a reaction between hydrogen peroxide and a coordination complex. These radicals attack the nucleic acids, resulting in the formation of aldehyde forms of ribose (in RNA) or deoxyribose (in DNA).

10. I have also studied the Ekenberg patent cited in the last Official Action. It should be stated up front that the primary objective of the Ekenberg patent is to isolate RNA only and treat all other nucleic acid material as waste material. This is because Ekenberg wishes to enhance the hybridization signal of isolated RNA by minimizing background noise otherwise caused by the remaining genetic detritus.

11. Ekenberg is not a labeling process. It is simply an RNA isolation process. No chromophores are employed in Ekenberg, and therefore no chromophore-receiving moieties need be prepared. This is why Ekenberg's process time is less than the *labeling* time of Proudnikov. Proudnikov and Ekenberg have different objectives.

12. The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any resulting patent.

IN WITNESS WHEREOF, I have signed, sealed, and delivered this instrument this fifth day of August, 2002.

8/5/02
Date

Sergei Bavykin, PhD.